Lenalidomide promotes the expansion of CD8 T cells with an effector memory phenotype in a murine xenograft model of chronic lymphocytic leukemia

Rita Simone PhD[1], Sonia Marsilio PhD[1] , Piers E.M. Patten MD PhD[2] , Gerardo Ferrer PhD[1] , Shih-Shih Chen PhD[1] , Yan Xiao Jie MD[1], Stefano Vergani Msc[1], Andrea Nicola Mazzarello PhD[1], Jacqueline Barrientos MD[3,4], Steven L. Allen MD[3,4] , Jonathan E. Kolitz MD[3,4] , Kanti R. Rai MD[1,3,4], and Nicholas Chiorazzi MD[1,3,4]

[1]The Feinstein Institute for Medical Research, North Shore - LIJ Health System, Manhasset, NY; [2]Department of Haemato-Oncology, King's College London, London, United Kingdom; [3]*Department of Medicine, North Shore University Hospital and Long Island Jewish Medical Center, North Shore - LIJ Health System, Manhasset and New Hyde Park, NY; [4]Department of Medicine, Hofstra North Shore-LIJ School of Medicine, Hempstead, NY, USA.*

Lenalidomide (Revlimid®), a thalidomide analogue, is an orally administered second generation immunomodulator with anti-angiogenic and anti-neoplastic properties. Initial studies treating patients with chronic lymphocytic leukemia (CLL) suggest that lenalidomide can have considerable efficacy and that its mode of action is mainly indirect, affecting non-malignant cells in the microenvironment, in particular T lymphocytes. Because a recently described xenograft model for CLL has highlighted the importance of CLL-derived, autologous T cells in promoting leukemic B-cell engraftment and growth in vivo, we have studied the influence of lenalidomide on the expansion of CLL B- and T-lymphocytes in this model.

After an initial 12 day culture of FACS-isolated CLL-derived T cells with or without anti-CD3/CD28 beads plus IL-2 (30 IU/ml), T lymphocytes were transferred into alymphoid NSG mice via the retro-orbital plexus (day 0). On day 7, CLL cells were delivered retro-orbitally. These recipient animals are referred to as “T + PBMC mice”. Mice that did not receive T cells on day 0 but were given CLL PBMCs at day 7, with or without lenalidomide, served as controls (“PBMC only mice”). Recipient mice received lenalidomide (10mg/kg/day) or vehicle control daily by gavage starting at day 0. All mice were sacrificed at day 28 (28 days after T-cell and 21 days after B-cell transfer), and blood, spleen, and bone marrow were collected. On this material, four analyses were performed: [1] level of human $CD45^+$ cell engraftment; [2] numbers and types of CLL-derived T cells; [3] numbers of CLL B cells; and [4] levels of cytokines reflective of Th1 and Th2 immune responses.

There was a clear enhancement in human hematopoietic ($CD45^+$) cell engraftment in those mice exposed to lenalidomide. This was most marked for the PBMC only mice (vehicle: 10.64%; lenalidomide: 38.53%), although it was also evident for T + PBMC mice (vehicle: 55.96%; lenalidomide: 69.65%).

T-cell phenotyping was carried out, before and after cell culture and also at sacrifice. Prior to culture, CLL samples contained on average ~96% $CD5^+CD19^+$ cells and ~3% $CD5^+CD19^-$ cells; for the latter, ~67% were $CD4^+$ and ~33% $CD8^+$. After 12-day culture, these percentages remained largely unchanged. However, the numbers and types of T cells recovered from the spleens at sacrifice were quite different after in vivo exposure to lenalidomide. For the PBMC only, the percentages of $CD4^+$ and $CD8^+$ cells in the spleens differed somewhat based on lenalidomide exposure (CD4: Vehicle 86% vs. Lenalidomide 61%; CD8: Vehicle 10% vs. Lenalidomide 28%). However, this change was dramatic for the T + PBMC mice (CD4: Vehicle 64.1% vs. Lenalidomide 28.9%; CD8: Vehicle 34% vs. Lenalidomide 62%). Furthermore, when the $CD8^+$ cells from these animals were subsetted based on antigen-experience and function, it appeared that lenalidomide exposure had led to the outgrowth of a greater number of effector memory ($CD45RO^+$ $CD62L^-$) than central memory ($CD45RO^+$ $CD62L^+$) T-cells.

For CLL-derived B cells, the numbers differed, based not only on lenalidomide exposure but also on prior in vitro activation. Specifically, in PBMC only mice, the addition of lenalidomide led to increased numbers of CLL B cells in the spleen (Vehicle: 7.81% vs. Lenalidomide: 14%). Conversely, in the T + PBMC mice, the numbers of B cells decreased (Vehicle: 2.36% vs. Lenalidomide: 0.34%).

An analysis of Th1 and Th2-related cytokines in the plasmas of the mice at sacrifice revealed a fall in IL-4, IL-5, and IL-10 and a marked increase in IFNγ, consistent with a Th2 to Th1 transition.

The above data suggest that administration of lenalidomide permits greater engraftment of human hematopoietic cells in alymphoid mice. Although this enhancement involves all members of the hematopoietic lineage, T cells, in particular $CD8^+$ effector memory T cells, emerge in excess over time. This CD8 expansion is associated with diminished levels of CLL B cells suggesting that the decrease is due to T-cell mediated cytolysis. In contrast, in the absence of prior T-cell activation, CLL T cells appear to support better CLL B-cell growth. These findings suggest that lenalidomide alters B-cell expansion in vivo depending on the activation and differentiation state of the autologous T-cell compartment. They also implicate the generation of cytolytic T cells as one mechanism whereby lenalidomide leads to clinical improvement in CLL.